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PATENT COOPERATION TREATY

PCT

REC'D 26 OCT 2000
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
00537/181WO1		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/14869	09/07/1999	23/07/1998
International Patent Classification (IPC) or national classification and IPC A61K38/16		
Applicant BIOMEASURE INCORPORATED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21/02/2000	24.10.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Schnack, A Telephone No. +49 89 2399 8149

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/14869

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-17,20,21	as originally filed				
18,19	as received on	29/08/2000	with letter of	29/08/2000	

Claims, No.:

1-48	as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule

68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-16.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-16
	No:	Claims	none
Inventive step (IS)	Yes:	Claims	none
	No:	Claims	1-16
Industrial applicability (IA)	Yes:	Claims	1-16
	No:	Claims	none

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Reference is made to the following document:

D1: US 5 445 832

Section I
Basis

In view of objections raised in the first written opinion concerning clarity of the present examples, applicant has amended pages 18 and 19. The amendment made on page 18, line 14, ("the above product" has been changed to "example 1(a)") can be allowed under Rule 70.2(c) PCT, because it was clearly meant to the peptide composition, which was added, since the peptide content was determined to 2%, (see page 18, lines 19-20). Also the amendments made on page 19 appears to be acceptable under Rule 70.2(c) PCT, the reason being as follows: applicant has originally under 1(g) stated that 1(b) and 1(d) were tested in-vivo, (see page 19, line 6, 7 and table 1). However, since 1(b) and 1(d) are not microsphere formulations, but rather intermediates, it was obviously not these formulations, which were tested in-vivo. The only final microsphere formulations are the ones originally designated 1(c) and 1(e). Thus, it appears to be clear that it was these formulations, which were tested and the amendments can therefore be accepted.

Section IV
Non-unity
Objections under Rule 13 PCT:

This IPEA finds that the present application contains three inventions, grouped as follows:

Claims 1-16:
A process for preparing polymer microspheres comprising a polymer and a peptide, which comprises among other steps, the steps of:

-suspending a peptide complex in an organic solvent
-dispersing the suspension in an aqueous solution
-evaporating the organic solvent

Claims 17-32:
A process for preparing polymer microspheres comprising a polymer and a peptide, which comprises among other steps, the steps of:

-dissolving a peptide complex in an organic solvent
-dispersing the solution in an aqueous solution of a surfactant
-evaporating the organic solvent

Claims 33-48:
A process for preparing polymer microspheres comprising a polymer and a peptide, which comprises among other steps, the steps of:

-dissolving a peptide complex in an organic solvent
-dispersing the solution in a surfactant
-evaporating the organic solvent

Thus, the only common technical relationship between present independent claims 1, 17 and 33 is that the peptide complex is:

-suspended/dissolved in an organic solvent
-dispersed in a medium (aqueous solution, aqueous solution of a surfactant and surfactant only, respectively)
-the organic solvent is evaporated.

However, these common technical features do not constitute a novel and inventive common concept in the meaning of Rule 13 PCT, because these features, i.e a process having these steps, are known from e.g. D1, (see D1, the abstract, col. 2, lines 8-21 and claims 1 and 2).

Thus, the applicant has requested preliminary examination of claims 1-16 without formal deletion of the remaining claims. Therefore, the present application still lacks unity and this report is thus based on only the claims 1-16 for which fees have been paid.

Section V
V.1. Novelty
Remarks under Article 33(2) PCT:

Present claim 1 is directed to a process for preparing microspheres, the process comprises the step of:

-neutralizing a peptide salt with a weak base in a aqueous medium containing hydroxyapatite or $CaHPO_4$ to form a precipitate

-isolating the precipitate

-suspending the precipitate in an organic solvent containing a polymer

-dispersing the suspension in an aqueous solution of a surfactant

-evaporating the organic solvent

D1 discloses a process for preparing microspheres. This process differs from the present process only in that the present claim 1 requires the presence of hydroxyapatite or $CaHPO_4$, whereas D1 is silent about such components, (see D1, the whole document and especially the passages indicated in the search report). Thus, the subject matter of present claims 1-16 appears to be novel with respect to D1.

V.2. Inventive step

Objections under Article 33(3) PCT:

D1, which can be considered to be the closest prior art, discloses a method for preparing microspheres, said method only differs from the present method in that hydroxyapatite or $CaHPO_4$ are not mentioned as means to enhance the encapsulation efficiency. D1 does teach that it is desirable to obtain the peptide in a water-insoluble form, (see D1, col. 1, line 61 - col. 2, line 4).

It is however unclear which technical effects the inclusion of hydroxyapatite or $CaHPO_4$ offer in comparison to the microspheres according to D1. The present application does not provide any comparative tests not does it appear to disclose in any other way that the presence of hydroxyapatite or $CaHPO_4$ offers advantages over prior art microspheres according to D1. Thus, it appears that an inventive step has not been shown for the present subject matter.

Applicants arguments that since D1 does not even refer to hydroxyapatite or $CaHPO_4$ and inventive step is *per se* present, cannot be accepted: If the presence of these two components does not offer any advantages over the microshperes according to D1, no technical effect can be ascribed such a presence. It further appears that the microspheres according to D1 have a good encapsulation efficiency and they elicit also plasma testosterone levels comparable with the present levels, (see D1, e.g. col. 5, lines 20-24 and lines 35-45). In other words, adding a technical feature, which does not have any technical effect, to prior art teaching cannot be considered to involve an inventive step. Thus, in order to demonstrate an inventive step of the present subject matter, it appears that it must be shown that the present microspheres have advantages, which are not possessed by the microspheres according to D1.

V.3. Industrial applicability

Remarks under Article 33(4) PCT:

The subject matter of present claims 1-16 fulfils the requirements for industrial applicability.

Section VIII

Objections under Article 6 PCT:

It appears that present example 1 is still unclear, because 1(e) states that the microshperes were prepared by employing the same procedure as 1(b). However, no microspheres were prepared in 1(b). Applicant probably means "1(b) and 1(c)".

by adding 1N $NaHCO_3$ dropwise. The precipitate was left stirring for about 2 hrs. The precipitate was collected by centrifugation. The precipitate was suspended in water and lyophilized.

Peptide content by nitrogen analysis = 23.6% and by HPLC= 22.1%.

1(b): Preparation of neutralized polyvinyl alcohol (PVA) solution

Commercially available PVA has pH lower than 5, due to the presence of hydrolysis product of poly(vinylacetate) from which PVA is prepared. The PVA solution was cleaned by preparing a concentrated solution in water, neutralizing with $NaHCO_3$ solution, dialyzing against de-ionized water. The neutralized PVA was precipitated in acetone, filtered and vacuum dried.

1(c): Preparation of p(dl-lactic acid) microspheres

1 g of p(dl-lactic acid) available from (Pharma-Biotech, ZI de Signes, BP 707, 83030 Toulon Cedex-9, France) (Mn= 32K, Mw= 54.4K) was dissolved in 10 ml DCM and 100 mg of the example 1(a) was suspended in the solution. The solution was cooled in an ice-bath and was dispersed in 100 ml of 1% pre-cooled PVA (polyvinyl alcohol) solution using a Polytron homogenizer (Kinematica, Switzerland). DCM was rotovaped and the microspheres were collected by centrifugation. The particles were suspended in water and lyophilized. Peptide content determined by nitrogen analysis was 2% (calculated 2.2%).

1(d): Preparation of neutralized Tryptorelin in presence of HAP

To 500 mg of acetate salt of pyroGlu-His-Trp-Ser-Tyr-DTrp-Leu-Arg-Pro-Gly-NH_2 (Kinerton, Dublin, Ireland) dissolved in 5 ml of water was added 200 mg of HAP. The pH of the solution was brought up to 7-8 using 1N $NaHCO_3$. The solution was left standing for about 2 hrs. and the precipitate was collected by centrifugation, and suspended in water and lyophilized. Peptide content by nitrogen analysis = 58.9%.

1(e): Preparation of microspheres containing 1(d)

Microspheres were prepared by employing the same procedure as 1(b). Peptide content 4.9%.

1(f): Co-precipitation of Tryptorelin and Calcium Phosphate monobasic

A solution of 100 mg of $CaHPO_4$ (Aldrich Chemicals, St. Louis, MO) and 100 mg of the acetate salt of pyroGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-

NH_2 (Kinerton, Dublin, Ireland) in water was prepared. The pH of the solution was brought to about 7 using 1N $NaHCO_3$ and left for about 24 hrs. for the completion of the precipitate. The precipitate was centrifuged, collected, suspended in water and lyophilized. Peptide content determined by HPLC method was 49.4%.

1(g): In-Vivo testing of 1(c) and 1(e) in rats

Formulations 1(c) & 1(e) were administered in male rats by IM injection at a dose of 300 µg of tryptorelin equivalent per rat, as a dispersion of the microspheres in 1% (w/v) Tween 20® (Aldrich Chemicals, St. Louis, MO) and 2% (w/v) carboxymethyl cellulose (Aldrich Chemicals, St. Louis, MO). The testosterone response was monitored by RIA: 50µL of the blood sample, 200µL of 125I-testosterone and 200µL of antiserum were poured into tubes which were shaken and incubated for 2 hrs. at 37°C. The immunoprecipitant reagent (1ml) was added to each tube and all the tubes were incubated for 15 minutes at room temperature. The supernatent was eliminated after centrifugation and the radioactivity was measured with LKB Wallace gamma counter.. The plasma testosterone levels are shown below.

Table 1

Plasma testosterone response (ng/ml) to IM injection of 300 µg of Tryptorelin equivalent/rat

Sample	6 h	Day 2	Day 3	Day 5	Day 10	Day 15	Day 23	Day 30	Day 37
1(c)	5.37	4.08	0.74	0.45	0.30	0.31	0.90	0.61	0.81
1(e)	5.32	3.58	1.04	0.29	0.38	0.56	0.80	0.76	0.72

Example 2

2(a): Preparation of water-insoluble salts of peptides with carboxylated p(dl-LGA)

Water insoluble salts of peptides with carboxy functionalized PLGA were prepared as described in US Patent No. 5,672,659 the teachings of which are incorporated herein by reference.

AMENDED SHEET

Q2

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
00537/181W01		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/ 14869	09/07/1999	23/07/1998
Applicant		
BIOMEASURE INCORPORATED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. --

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 99/14869

A. CLASSIFICATION OF SUBJECT MATTER

A61K38/16, A61K9/113, C07K17/02, C07K1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K, C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5445832 A (ORSOLINI ET AL.) 29 August 1995, abstract, column 3, lines 1, 2,18-23,37-45, example 1, claims.	1,6-11,14, 15,17-21,30, 33-37, 46
X	PATENT ABSTRACTS OF JAPAN, vol. 16, no. 207, 18 May 1992; & JP 04 036233 A (BIOMATERIAL UNIVERSE K.K.), 06 February 1992, abstract.	1,3,14
A	US 5160745 A (DE LUCA ET AL.) 03 November 1992, abstract, claims.	1-48

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search
04 November 1999

Date of mailing of the international search report
27. 12. 1999

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer
KRENN e.h.

Form PCT/ISA/210 (second sheet) (July 1992)

International Application No
PCT/US 99/14869

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5578709 A (WOISZWILLO, J.E.) 26 November 1996, abstract, claims.	1-48

ANHANG

zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.

ANNEX

to the International Search Report to the International Patent Application No.

ANNEXE

au rapport de recherche international relatif à la demande de brevet international n°

PCT/US 99/14869 SAE 242610

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The Office is in no way liable for these particulars which are given merely for the purpose of information.

La présente annexe indique les membres de la famille de brevets relatifs aux documents de brevets cités dans le rapport de recherche international visée ci-dessus. Les renseignements fournis sont donnés à titre indicatif et n'engagent pas la responsibilité de l'Office.

Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
US A 5445832	29-08-1995	AT A 1489/92	15-06-1997
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			PT	B	30-07-1999
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			SE	A0	21-07-1992
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			SI	A	31-03-1993
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			AU	B2	24-08-1990
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			GR	T3	31-05-1996
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			AU	A1	26-09-1994
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			US	A	15-12-1998
			WO	A2	04-07-1996
			WO	A3	13-02-1997

PATENT COOPERATION TREATY

4 RT

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

TSAO, Y. Rocky
FISH & RICHARDSON P.C.
225 Franklin Street
Boston, MA 02110-2804
ETATS-UNIS D'AMERIQUE

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OCT 30 2000
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BOSTON OFFICE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

	Date of mailing *(day/month/year)* 24.10.2000
Applicant's or agent's file reference 00537/181WO1	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/14869	09/07/1999	23/07/1998

Applicant
BIOMEASURE INCORPORATED et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

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(71) Applicant *(for all designated States except US)*: BIOMEASURE INCORPORATED [US/US]; 27 Maple Street, Milford, MA 01757–3650 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: IGNATIOUS, Francis, X. [IN/US]; 15 Eagle Rock Drive, Millville, MA 01529 (US).

(74) Agent: TSAO, Y., Rocky; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110–2804 (US).

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(57) Abstract

This invention relates to a process for preparing biodegradable microspheres and/or nanospheres using an oil–in–water process for the controlled release of bioactive peptides.

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ENCAPSULATION OF WATER SOLUBLE PEPTIDES

Background of the Invention

This invention relates to a process for preparing biodegradable microspheres and or nanospheres using an oil-in-water process, which microspheres and nanospheres can be used for the controlled release of bioactive peptides.

A variety of techniques are described in the literature for the preparation of polymer microspheres for the sustained release of bioactive peptides. Among the different techniques such as spray drying, spray congealing, coacervation, solvent evaporation etc., solvent evaporation is simplest to scale-up industrially (for a recent review see protein delivery from biodegradable microspheres, by J.L. Cleland in Protein Delivery edited by L. Sanders and W. Hendren, Plenum Press, NY 1997). Solvent evaporation is usually practiced by dissolving or suspending an active ingredient in a polymer solution, which is further dispersed in the form of droplets in a suitable medium containing surfactants capable of stabilizing the droplets, and the polymer droplets are hardened by evaporation of the solvent. When the polymer is dissolved in an organic medium and then emulsified in water, the process is called oil-in-water process (O/W). Water soluble peptides cannot be encapsulated by the O/W process, due to the partition of the water soluble peptides into the aqueous medium, resulting in low encapsulation efficiency. Higher encapsulation efficiencies were achieved by a more complex double emulsion water-in-oil-in-water (W/O/W) process (US Patent No. 5,271,945) or by using an oil-in-oil (O/O) process (EP 0330180 B1). The main drawback of the latter process is the use of different organic solvents, first to solubilize the polymer, and then to wash the polymer microspheres free of the oil in which they are formed. Therefore, the simple O/W emulsion solvent evaporation process is the most attractive, provided higher encapsulation efficiency can be achieved, since only one organic solvent is involved, and the residual organic solvent can be removed by vacuum drying.

The main hurdle to achieving higher encapsulation efficiency of the peptides is their water solubility. Solubility of peptides depends on the nature of

the counter-ion. The aqueous solubility of a peptide is considerably reduced when the peptide is present as a free base, due to intermolecular interactions. One method of enhancing the encapsulation efficiency of the peptides in an O/W process according to the present invention, is by using a peptide as a free base adsorbed onto a bioresorbable inorganic matrix, such as hydroxyapatite, Calcium monohydrogen phosphate, zinc hydroxide, alum etc. In the case of encapsulation of LHRH agonists such as tryptorelin, leuprolin, goserlin, busrelin, etc., the presence of calcium phosphate in the micropheres may not only serve to stabilize the neutralized peptide but also act as a calcium supplement, since one of the biggest concerns of continuous therapy using LHRH agonists is loss of bone density. This method of encapsulation is most suited when the peptide loading in excess of 5-6% is not desired. In the case of high peptide loading, a heterogeneous distribution of the drug particles, even if they were stabilized by adsorption onto a solid matrix or not, inside the microspheres leads to non-predictable release profiles.

In cases where higher drug loading as well as predictable release profiles are desired, a second method of reducing the aqueous solubility of the drug, without sacrificing its potency, is by simply forming reversible water insoluble salts of mono-functional or multi-functional detergents and/or polymers or a combination of both, as exemplified by Schally et al. in US Patent No. 4,010,125. The aqueous solubility of the peptides can be considerably reduced by forming salts of mono-functional detergents such as sodium dodecyl sulfate, or of multi-functional anionic species such as pamoate, tannate, alginate, carboxymethyl cellulose, leading to the precipitation of the water insoluble peptide salt. Among the water insoluble salts, some exhibit good solubility in common organic solvents. U.S. Patent No. 5,672,659 describes compositions formed between anionic carboxylate functionalized polyesters and cationic peptides. These compositions as well as those formed with certain anionic detergents such as dioctylsulfosuccinate are found to exhibit good solubility in organic solvents such as dichloromethane (DCM), chloroform, acetonitrile, ethyl acetate, and the like.

During the water based encapsulation of the peptide, either as a free base adsorbed on to solid matrix or as water insoluble but organic solvent

soluble salt, the pH of the aqueous medium can dramatically increase the water solubility, by affecting the equilibrium between the complexed and uncomplexed state. If the pH is not maintained at 7 the equilibrium may shift, favoring the solubilization of the peptide, leading to poor encapsulation efficiency.

It is therefore the object of the present invention to provide polymer microspheres and/or nanospheres prepared by a simple O/W method, where the encapsulation efficiency achieved can be greater than 85%.

Summary of the invention

In one aspect, the present invention is directed to process A, which is a process for preparing polymer microspheres comprising a polymer and a peptide, which comprises the steps of:

neutralizing a peptide salt with a weak base in an aqueous medium wherein said medium comprises a suspension of hydroxyapatite or a solution of calcium mono-hydrogen phosphate to form a precipitate;

isolating the precipitate;

suspending the precipitate in an organic solvent, which comprises a polymer dissolved therein to form a suspension;

dispersing the suspension in an aqueous solution of a surfactant; and

evaporating the organic solvent to isolate the polymer microspheres.

A preferred process of process A, comprises the additional step of dissolving the peptide salt in a minimum of water before neutralizing the peptide salt.

In a second aspect, the present invention is directed to process B, which is a process for preparing polymer microspheres and nanospheres comprising a polymer and a peptide, which comprises the steps of:

dissolving a salt of a peptide complexed with an anionically or cationically functionalized biodegradable polyester in an organic solvent to form a solution;

dispersing the solution in an aqueous solution of a surfactant; and

evaporating the organic solvent to isolate the polymer microspheres and nanospheres.

A preferred process of process B is where the anionically functionalized biodegradable polyester is functionalized with an anionic moiety selected from the group consisting of carboxylate, phosphate and sulfate and the cationically

functionalized biodegradable polyester is functionalized with a cationic moiety selected from the group consisting of amino, amidino, guadino, ammonium, cyclic amino groups and nucleic acid bases.

In a third aspect, the present invention is directed to a process for preparing polymer microspheres and nanospheres comprising a polymer and a peptide, which comprises the steps of:

dissolving a salt of a peptide complexed with an anionic counterion in an organic solvent which is selected from the group consisting of dichloromethane, chloroform and ethyl acetate to form a solution;

dispersing the solution in a surfactant; and

evaporating the organic solvent to isolate the polymer microspheres and nanospheres.

A preferred process of any of the foregoing processes is where the surfactant is one or more of sodium oleate, sodium stearate, sodium laurylsulphate, a poly(oxyethylene) sorbitan fatty acid ester, polyvinylpyrrolidine, polyvinyl alcohol, carboxymethyl cellulose, lecithin, gelatin or hyaluronic acid.

A preferred process of any of the foregoing processes is where the surfactant is polyvinyl alcohol and the pH of the polyvinyl alcohol is 6.5-7.5.

A preferred process of any of the foregoing processes is where the pH of the polyvinyl alcohol is 6.9-7.1.

A preferred process of any of the foregoing processes is where the organic solvent is dichloromethane, chloroform or ethyl acetate.

A preferred process of any of the foregoing processes is where the organic solvent is dichloromethane and the concentration of the polymer in dichloromethane is 0.5% to 30% by weight.

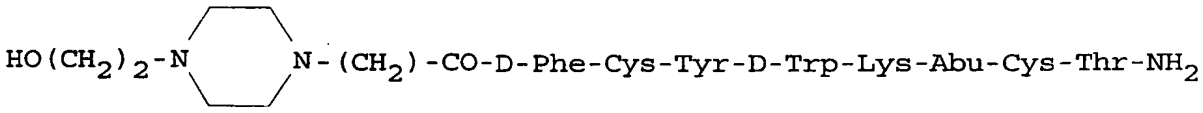
A preferred process of any of the foregoing processes is where the concentration of the polymer in dichloromethane is 0.5% to 10% by weight.

A preferred process of any of the foregoing processes is where the peptide is growth hormone releasing peptide, luteinizing hormone-releasing hormone, somatostatin, bombesin, gastrin releasing peptide, calcitonin, bradykinin, galanin, melanocyte stimulating hormone, growth hormone releasing factor, amylin, tachykinins, secretin, parathyroid hormone, enkephalin, endothelin, calcitonin gene releasing peptide, neuromedins, parathyroid

hormone related protein, glucagon, neurotensin, adrenocorticothrophic hormone, peptide YY, glucagon releasing peptide, vasoactive intestinal peptide, pituitary adenylate cyclase activating peptide, motilin, substance P, neuropeptide Y, or TSH or an analogue or a fragment thereof or a pharmaceutically acceptable salt thereof.

A preferred process of any of the foregoing processes is where the peptide is the LHRH analogue of the formula pyroGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2.

A preferred process of any of the foregoing processes is where the peptide is selected from the group of somatostatin analogues consisting of H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-NH_2,



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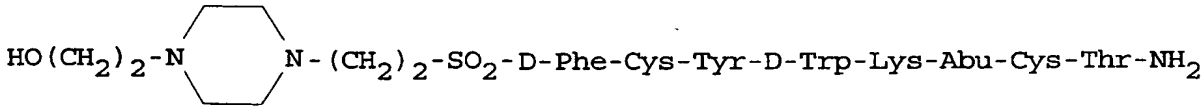

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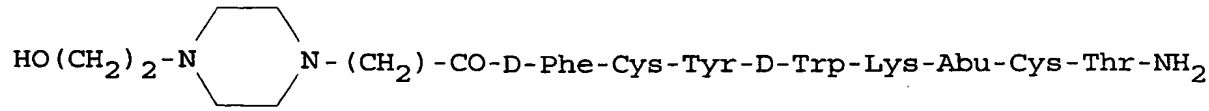
A preferred process of any of the foregoing processes is where the polymer is polylactide-co-glycolide, polycaprolactone or polyanhydride or a copolymer or blends thereof.

In another aspect, the present invention is directed to a polymer microsphere made according to process A, process B or process C.

Preferred of the immediately foregoing process is where the polymer is polylactide-co-glycolide, polycaprolactone or polyanhydride or a copolymer or blends thereof and where the peptide is the LHRH analogue of the formula pyroGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2 or the peptide is selected from the group of somatostatin analogues consisting of H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-NH_2,



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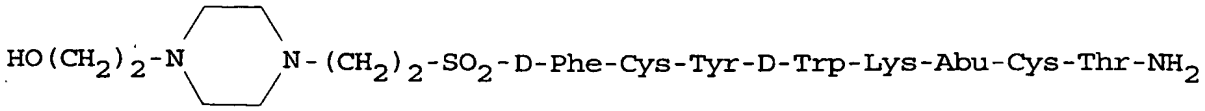


Detailed Description of the Invention

The terms biodegradable and bioerodable are used interchangeably and is intended to mean that the material is degraded in the biological environment of the subject that to which it is administered.

Polymer microspheres made according to a process of this invention can be administered by intramuscular (IM), subcutaneous, pulmonary or oral route. Polymer nanospheres made according to a process of this invention in addition to being deliverable in the same manner as disclosed for microspheres can also be administered via inhalation methods such as those discussed in *Pulmonary Drug Delivery*, J. Yu and Y.W. Chien in Critical Reviews™ in Therapeutic Drug Carrier Systems, 14(4): 395-453, (1997), the contents of which are incorporated herein by reference. The microspheres and nanospheres made according to a process of this invention contain from less than 0.1% by weight up to approximately 50% by weight of a peptide. The polymer microspheres containing a peptide are prepared by an O/W emulsion solvent evaporation process, without compromising the much desired high encapsulation efficiency. Encapsulation efficiencies greater than 85% can be achieved according to the teachings of the present invention.

Polymers that can be used to form microspheres include bioerodible polymers such as polyesters (ex. polylactides, polyglycolides, polycaprolactone and copolymers and blends thereof), polycarbonates, polyorthoesters, polyacetals, polyanhydrides, their copolymers or blends, and non-bioerodible polymers such as polyacrylates, polystyrenes, polyvinylacetates, etc. Both types of polymers may optionally contain anionic or cationic groups. In general a polymer solution can be prepared containing between 1% and 20% polymer, preferably between 5% and 15% polymer. The polymer solution can be prepared in dichloromethane (DCM), chloroform, ethylacetate, methylformate, dichloroethane, toluene, cyclohexane and the like.

Any peptide can be incorporated in the microspheres of this invention. Examples of peptides that can be incorporated in the microspheres produced by a process of this invention are growth hormone releasing peptide (GHRP), luteinizing hormone-releasing hormone (LHRH), somatostatin, bombesin, gastrin releasing peptide (GRP), calcitonin, bradykinin, galanin, melanocyte stimulating hormone (MSH), growth hormone releasing factor (GRF), amylin, tachykinins, secretin, parathyroid hormone (PTH), enkephalin, endothelin, calcitonin gene releasing peptide (CGRP), neuromedins, parathyroid hormone related protein (PTHrP), glucagon, neurotensin, adrenocorticothrophic hormone (ACTH), peptide YY (PYY), glucagon releasing peptide (GLP), vasoactive intestinal peptide (VIP), pituitary adenylate cyclase activating peptide (PACAP), motilin, substance P, neuropeptide Y (NPY), TSH and analogs and fragments thereof or a pharmaceutically acceptable salt thereof.

The term "peptide" is intended to include peptide, polypeptides and proteins.

Examples of specific LHRH analogues that can be incorporated in the microspheres of this invention are tryptorelin (p-Glu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2), buserelin ([D-Ser(t-Bu)^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), deslorelin ([D-Trp^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt, fertirelin ([des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), gosrelin ([D-Ser(t-Bu)^6, Azgly^{10}]-LHRH), histrelin ([D-His(Bzl)^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), leuprorelin ([D-Leu^6, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), lutrelin ([D-Trp^6, MeLeu^7, des-Gly-NH_2^{10}]-LHRH(1-9)NHEt), nafarelin ([D-Nal^6]-LHRH and pharmaceutically acceptable salts thereof.

Preferred somatostatin analogs that can be incorporated in the microspheres and/or nanospheres of this invention are those covered by formulae or those specifically recited in the publications set forth below, all of which are hereby incorporated by reference:

Van Binst, G. et al. Peptide Research 5:8 (1992);

Horvath, A. et al. Abstract, "Conformations of Somatostatin Analogs Having Antitumor Activity", 22nd European peptide Symposium, September 13-19, 1992, Interlaken, Switzerland;

PCT Application WO 91/09056 (1991);

EP Application 0 363 589 A2 (1990);

U.S. Patent No. 4,904,642 (1990);
U.S. Patent No. 4,871,717 (1989);
U.S. Patent No. 4,853,371 (1989);
U.S. Patent No. 4,725,577 (1988);
U.S. Patent No. 4,684,620 (1987)
U.S. Patent No. 4,650,787 (1987);
U.S. Patent No. 4,603,120 (1986);
U.S. Patent No. 4,585,755 (1986);
EP Application 0 203 031 A2 (1986);
U.S. Patent No. 4,522,813 (1985);
U.S. Patent No. 4,486,415 (1984);
U.S. Patent No. 4,485,101 (1984);
U.S. Patent No. 4,435,385 (1984);
U.S. Patent No. 4,395,403 (1983);
U.S. Patent No. 4,369,179 (1983);
U.S. Patent No. 4,360,516 (1982);
U.S. Patent No. 4,358,439 (1982);
U.S. Patent No. 4,328,214 (1982);
U.S. Patent No. 4,316,890 (1982);
U.S. Patent No. 4,310,518 (1982);
U.S. Patent No. 4,291,022 (1981);
U.S. Patent No. 4,238,481 (1980);
U.S. Patent No. 4,235,886 (1980);
U.S. Patent No. 4,224,190 (1980);
U.S. Patent No. 4,211,693 (1980);
U.S. Patent No. 4,190,648 (1980);
U.S. Patent No. 4,146,612 (1979);
U.S. Patent No. 4,133,782 (1979);
U.S. Patent No. 5,506,339 (1996);
U.S. Patent No. 4,261,885 (1981);
U.S. Patent No. 4,728,638 (1988);
U.S. Patent No. 4,282,143 (1981);
U.S. Patent No. 4,215,039 (1980);

U.S. Patent No. 4,209,426 (1980);
U.S. Patent No. 4,190,575 (1980);
EP Patent No. 0 389 180 (1990);
EP Application No. 0 505 680 (1982);
EP Application No. 0 083 305 (1982);
EP Application No. 0 030 920 (1980);
PCT Application No. WO 88/05052 (1988);
PCT Application No. WO 90/12811 (1990);
PCT Application No. WO 97/01579 (1997);
PCT Application No. WO 91/18016 (1991);
U.K. Application No. GB 2,095,261 (1981); and
French Application No. FR 2,522,655 (1983).

Examples of somatostatin analogs include, but are not limited to, the following somatostatin analogs which are disclosed in the above-cited references:

H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-NH_2;
H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-β-Nal-NH_2;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-β-Nal-NH_2;
H-D-β-Nal-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-NH_2;
H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Pen-Thr-NH_2;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-Thr-OH;
H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Pen-Thr-OH;
H-Gly-Pen-Phe-D-Trp-Lys-Thr-Cys-Thr-OH;
H-Phe-Pen-Tyr-D-Trp-Lys-Thr-Cys-Thr-OH;
H-Phe-Pen-Phe-D-Trp-Lys-Thr-Pen-Thr-OH;
H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol;
H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;
H-D-Trp-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;
H-D-Trp-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;
H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;

Ac-D-Phe-Lys*-Tyr-D-Trp-Lys-Val-Asp*-Thr-NH_2 (an amide bridge formed between Lys* and Asp*);

Ac-hArg$(Et)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(Et)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg(Bu)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(Et)_2$-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-L-hArg$(Et)_2$-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(CH_2CF_3)_2$-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(CH_2CF_3)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(CH_2CF_3)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Phe-NH_2;

Ac-D-hArg$(CH_2CF_3)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NHEt;

Ac-L-hArg$(CH_2$-$CF_3)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(CH_2CF_3)_2$-Gly-Cys-Phe-D-Trp-Lys(Me)-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(CH_2CF_3)_2$-Gly-Cys-Phe-D-Trp-Lys(Me)-Thr-Cys-Thr-NHEt;

Ac-hArg(CH_3, hexyl)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

H-hArg$(hexyl_2)$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(Et)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NHEt;

Ac-D-hArg$(Et)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Phe-NH_2;

Propionyl-D-hArg$(Et)_2$-Gly-Cys-Phe-D-Trp-Lys(iPr)-Thr-Cys-Thr-NH_2;

Ac-D-β-Nal-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Gly-hArg$(Et)_2$-NH_2;

Ac-D-Lys(iPr)-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(CH_2CF_3)_2$-D-hArg$(CH_2CF_3)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-D-hArg$(CH_2CF_3)_2$-D-hArg$(CH_2CF_3)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Phe-NH_2;

Ac-D-hArg$(Et)_2$-D-hArg$(Et)_2$-Gly-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2;

Ac-Cys-Lys-Asn-4-Cl-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Ser-D-Cys-NH_2;

H-Bmp-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;

H-Bmp-Tyr-D-Trp-Lys-Val-Cys-Phe-NH_2;

H-Bmp-Tyr-D-Trp-Lys-Val-Cys-p-Cl-Phe-NH_2;

H-Bmp-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH_2;

H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-β-Nal-NH_2;

H-pentafluoro-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2;

Ac-D-β-Nal-Cys-pentafluoro-Phe-D-Trp-Lys-Val-Cys-Thr-NH_2;

H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-β-Nal-NH_2;

H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH_2;

H-D-p-Cl-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH_2;

Ac-D-p-Cl-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Thr-NH_2;

H-D-Phe-Cys-β-Nal-D-Trp-Lys-Val-Cys-Thr-NH_2;

H-D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2;

cyclo(Pro-Phe-D-Trp-N-Me-Lys-Thr-Phe);

cyclo(Pro-Phe-D-Trp-N-Me-Lys-Thr-Phe);

cyclo(Pro-Phe-D-Trp-Lys-Thr-N-Me-Phe);

cyclo(N-Me-Ala-Tyr-D-Trp-Lys-Thr-Phe);

cyclo(Pro-Tyr-D-Trp-Lys-Thr-Phe);

cyclo(Pro-Phe-D-Trp-Lys-Thr-Phe);

cyclo(Pro-Phe-L-Trp-Lys-Thr-Phe);

cyclo(Pro-Phe-D-Trp(F)-Lys-Thr-Phe);

cyclo(Pro-Phe-Trp(F)-Lys-Thr-Phe);

cyclo(Pro-Phe-D-Trp-Lys-Ser-Phe);

cyclo(Pro-Phe-D-Trp-Lys-Thr-p-Cl-Phe);

cyclo(D-Ala-N-Me-D-Phe-D-Thr-D-Lys-Trp-D-Phe);

cyclo(D-Ala-N-Me-D-Phe-D-Val-Lys-D-Trp-D-Phe);

cyclo(D-Ala-N-Me-D-Phe-D-Thr-Lys-D-Trp-D-Phe);

cyclo(D-Abu-N-Me-D-Phe-D-Val-Lys-D-Trp-D-Tyr);

cyclo(Pro-Tyr-D-Trp-t-4-AchxAla-Thr-Phe);

cyclo(Pro-Phe-D-Trp-t-4-AchxAla-Thr-Phe);

cyclo(N-Me-Ala-Tyr-D-Trp-Lys-Val-Phe);

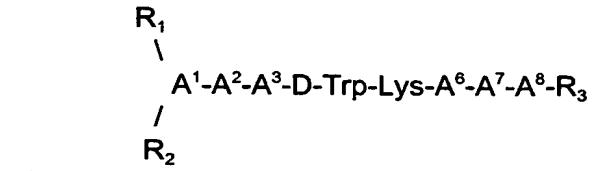
cyclo(N-Me-Ala-Tyr-D-Trp-t-4-AchxAla-Thr-Phe);

cyclo(Pro-Tyr-D-Trp-4-Amphe-Thr-Phe);

cyclo(Pro-Phe-D-Trp-4-Amphe-Thr-Phe);
cyclo(N-Me-Ala-Tyr-D-Trp-4-Amphe-Thr-Phe);
cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);
cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba-Gaba);
cyclo(Asn-Phe-D-Trp-Lys-Thr-Phe);
cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-$NH(CH_2)_4CO$);
cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-β-Ala);
cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-D-Glu)-OH;
cyclo(Phe-Phe-D-Trp-Lys-Thr-Phe);
cyclo(Phe-Phe-D-Trp-Lys-Thr-Phe-Gly);
cyclo(Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);
cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gly);
cyclo(Asn-Phe-Phe-D-Trp(F)-Lys-Thr-Phe-Gaba);
cyclo(Asn-Phe-Phe-D-Trp(NO_2)-Lys-Thr-Phe-Gaba);
cyclo(Asn-Phe-Phe-Trp(Br)-Lys-Thr-Phe-Gaba);
cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Phe(I)-Gaba);
cyclo(Asn-Phe-Phe-D-Trp-Lys-Thr-Tyr(But)-Gaba);
cyclo(Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Pro-Cys)-OH;
cyclo(Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Pro-Cys)-OH;
cyclo(Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-Tpo-Cys)-OH;
cyclo(Bmp-Lys-Asn-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-MeLeu-Cys)-OH;
cyclo(Phe-Phe-D-Trp-Lys-Thr-Phe-Phe-Gaba);
cyclo(Phe-Phe-D-Trp-Lys-Thr-Phe-D-Phe-Gaba);
cyclo(Phe-Phe-D-Trp(5F)-Lys-Thr-Phe-Phe-Gaba);
cyclo(Asn-Phe-Phe-D-Trp-Lys(Ac)-Thr-Phe-NH-$(CH_2)_3$-CO);
cyclo(Lys-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);
cyclo(Lys-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);
cyclo(Orn-Phe-Phe-D-Trp-Lys-Thr-Phe-Gaba);
H-Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys-NH_2;
H-Cys-Phe-Phe-D-Trp-Lys-Ser-Phe-Cys-NH_2;
H-Cys-Phe-Tyr-D-Trp-Lys-Thr-Phe-Cys-NH_2; and
H-Cys-Phe-Tyr(I)-D-Trp-Lys-Thr-Phe-Cys-NH_2.

A disulfide bridge is formed between the two free thiols (e.g., Cys, Pen, or Bmp residues) when they are present in a peptide; however, the disulfide bond is not shown.

Also included are somatostatin agonists of the following formula:



wherein

A^1 is a D- or L- isomer of Ala, Leu, Ile, Val, Nle, Thr, Ser, β-Nal, β-Pal, Trp, Phe, 2,4-dichloro-Phe, pentafluoro-Phe, p-X-Phe, or o-X-Phe, wherein X is CH_3, Cl, Br, F, OH, OCH_3 or NO_2;

A^2 is Ala, Leu, Ile, Val, Nle, Phe, β-Nal, pyridyl-Ala, Trp, 2,4-dichloro-Phe, pentafluoro-Phe, o-X-Phe, or p-X-Phe, wherein X is CH_3, Cl, Br, F, OH, OCH_3 or NO_2;

A^3 is pyridyl-Ala, Trp, Phe, β-Nal, 2,4-dichloro-Phe, pentafluoro-Phe, o-X-Phe, or p-X-Phe, wherein X is CH_3, Cl, Br, F, OH, OCH_3 or NO_2;

A^6 is Val, Ala, Leu, Ile, Nle, Thr, Abu, or Ser;

A^7 is Ala, Leu, Ile, Val, Nle, Phe, β-Nal, pyridyl-Ala, Trp, 2,4-dichloro-Phe, pentafluoro-Phe, o-X-Phe, or p-X-Phe, wherein X is CH_3, Cl, Br, F, OH, OCH_3 or NO_2;

A^8 is a D- or L-isomer of Ala, Leu, Ile, Val, Nle, Thr, Ser, Phe, β-Nal, pyridyl-Ala, Trp, 2,4-dichloro-Phe, pentafluoro-Phe, p-X-Phe, or o-X-Phe, wherein X is CH_3, Cl, Br, F, OH, OCH_3 or NO_2;

each R_1 and R_2, independently, is H, lower acyl or lower alkyl; and R_3 is OH or NH_2; provided that at least one of A^1 and A^8 and one of A^2 and A^7 must be an aromatic amino acid; and further provided that A^1, A^2, A^7 and A^8 cannot all be aromatic amino acids.

Examples of linear agonists to be used in a process of this invention include:

H-D-Phe-p-chloro-Phe-Tyr-D-Trp-Lys-Thr-Phe-Thr-NH_2;

H-D-Phe-p-NO_2-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2;

H-D-Nal-p-chloro-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2;

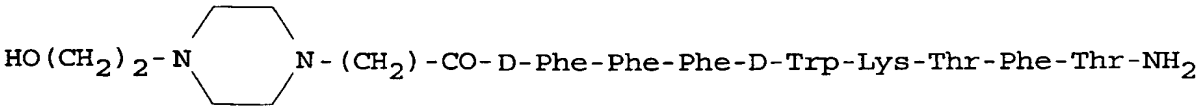
H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH_2;

H-D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2;

H-D-Phe-p-chloro-Phe-Tyr-D-Trp-Lys-Val-Phe-Thr-NH_2; and

H-D-Phe-Ala-Tyr-D-Trp-Lys-Val-Ala-β-D-Nal-NH_2.

If desired, one or more chemical moieties, e.g., a sugar derivative, mono or poly-hydroxy C_{2-12} alkyl, mono or poly-hydroxy C_{2-12} acyl groups, or a piperazine derivative, can be attached to the somatostatin agonist, e.g., to the N-terminus amino acid. See PCT Application WO 88/02756, European Application 0 329 295, and PCT Application No. WO 94/04752. An example of somatostatin agonists which contain N-terminal chemical substitutions are:



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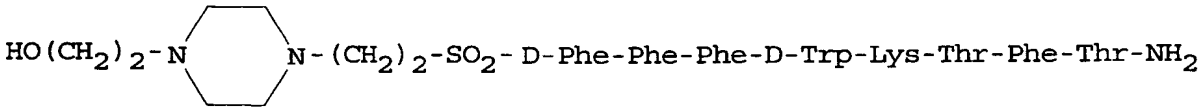

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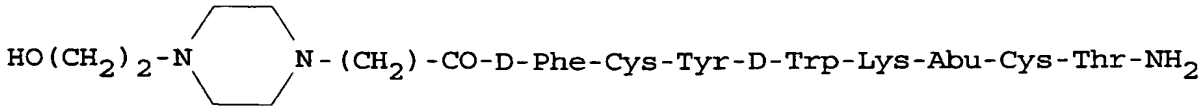
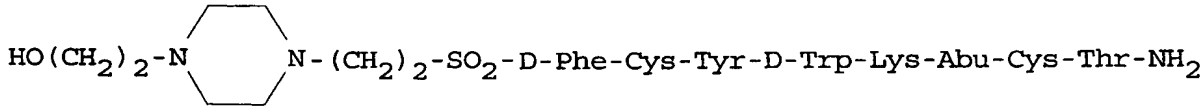


; and



Processes for making polymer microspheres and/or nanospheres according to a method of the present invention are described herein. The examples are given for illustrative purposes and are not meant to limit the scope of the present invention. All references cited herein are incorporated herein by reference.

Water solubility can be considerably diminished by co-precipitating the peptide as free base along with an inorganic bioresorbable matrix such as hydroxyapatite, calcium phosphate, alum, zinc hydroxide, etc. The presence of the inorganic bioresorbable matrix stabilizes the free, neutralized peptide by a combination of phenomena such as complexation, adsorption and the like.

The water insoluble peptide in the neutralized and adsorbed form can be prepared by dissolving a water soluble salt of a peptide such as acetate, trifluoroacetate, hydrochloride, sulphate, and the like, in a minimum amount of water and suspending hydroxyapatite in the solution, followed by addition of a weak base such as $NaHCO_3$, triethylamine, and the like to bring the pH up to 7-8. The precipitate so formed is filtered, suspended in water and lyophilized.

Another method of decreasing the water solubility of the peptide is by the formation of salts or complexes with either mono- or multi- functional, monomeric or polymeric counterions, such as dodecylsulfate, bisphosphonates, phosphatidyl inisitol, phosphorylated, sulfated or carboxylated cyclodextrins, alginates, carboxymethyl cellulose, dioctylsulfosuccinates, tannates, anionically functionalized polyesters, polycarbonates, polyesters, polyanhydrides, polyethers, polyorthoesters, present as their copolymers or blends, and the anionic functionality may be carboxylate, phosphate or sulfate, and the like. The nature of an anionic group present in the counter-ion complex influences the water solubility of a peptide by displacing the equilibrium between the complexed and uncomplexed peptide. This equilibrium constant depends on the acidity of the anionic functionality which decreases in order sulphate> phosphate> carboxylate.

Water insoluble peptide salts or complexes of the present invention may be prepared by adding an equivalent amount of a salt containing the desired counterion, such as sodium dodecylsulfate, sodium tannate, sodium pamoate, sodium dioctylsulfosuccinate, sodium alginate, sodium cyclodextrin sulfate, sodium cyclodextrin phosphate and the like, in water to an aqueous peptide solution. The precipitated peptide complex is centrifuged, collected and suspended in water and lyophilized.

Polymers that can be used to form microspheres include biodegradable polymers such as polyesters (ex. polylactides, polyglycolides, polycaprolactone and copolymers and blends thereof) polycarbonates, polyorthoesters, polyacetals, polyanhydrides, their copolymers or blends, and non-biodegradable polymers such as polyacrylates, polystyrenes, polyvinylacetates, etc. The biodegradable polymers are intended to degrade under physiological conditions over a period of time, to yield natural metabolites, such that the implant or the

depot does not require to be retrieved once the drug is exhausted. These polymers may optionally contain anionic or cationic groups. The anionic groups present in the polymer may be sulphate, phosphate, or carboxylate, capable of forming salts with basic bioactive substances. The polymers can be endowed with cationic functionalities (or basic groups), such as amino, amidino, guadino, ammonium, cyclic amino groups and nucleic acid bases, which can form salts with acidic bioactive molecules. In general a polymer solution can be prepared in a water immiscible organic solvent, containing between 1% and 20% polymer, preferably between 5% and 15%.

The polymer solution can be prepared in water immiscible organic solvents such as dichloromethane (DCM), chloroform, dichloroethane, trichloroethane, cyclohexane, benzene, toluene, ethyl acetate, and the like, which can be used alone or as a mixture thereof.

The polymer microspheres of the invention are made by either suspending or dissolving the coprecipitates, salts or complexes in a polymer solution, and emulsifying this mixture/solution in aqueous medium containing a surfactant.

Emulsification of the oil droplets in aqueous medium is performed by known methods of dispersion. The dispersion methods include the use of mixers such as propeller mixer, turbine mixer, colloid mill method, the homogenizer method, and the ultrasonic irradiation method.

The emulsification of the organic layer is done in an aqueous layer containing an emulsifier, which can stabilize O/W emulsions, such as anionic surfactants (sodium oleate, sodium stearate, sodium laurylsulphate, and the like), non-ionic surfactants such as poly(oxyethylene) sorbitan fatty acid esters like Tween 20®, Tween 60®, Tween 80®, polyvinylpyrrolidone, polyvinyl alcohol, carboxymethyl cellulose, lecithin, gelatin, hyaluronic acid and the like, which may be used separately or in combination. The amount used may be chosen appropriately from a range of about 0.01% to 20%, preferably about 0.05% to 10%.

One important aspect of the present invention is the role of the pH of the aqueous surfactant medium in which the emulsion droplets are formed, in

partitioning the peptide into the aqueous medium, thereby reducing the encapsulation efficiency. Encapsulation efficiency is the amount of peptide actually present in the microspheres compared to the amount initially used in the process. The peptide loss to the aqueous medium can be minimized by maintaining the pH of the aqueous medium between 6-8, preferably around 7.

Removal of the solvent in the oil phase is performed by any method known in the art: solvent removal may be effected by gradual reduction of pressure by stirring with a propeller type stirrer or a magnetic stirrer, or by adjusting the degree of vacuum with a rotary evaporator.

Microspheres and/or nanospheres formed by the removal of the solvent are collected by centrifugation or by filtration, followed by several repetitions of washing with deionized water to remove emulsifier and any unencapsulated peptide.

The washed microspheres are collected by filtration and dried under vacuum at about 30° C for about 24-48 hrs., in order to remove the residual solvent.

The peptide content of a microspheres and/or nanospheres made according to a process of this was determined by nitrogen analysis and also by HPLC method. In the HPLC method, about 20 mg of the sample dissolved in 0.1% TFA solution, was analyzed using a C_{18} column, using eluants A (0.1% TFA) and eluant B (80% acetonitrile, 0.1% TFA), programmed at a gradient of 20% to 80% B in 50 min, and the peptide was monitored at 280nm by a UV detector (Applied Biosystems, Model # 785A). The HPLC system consisted of two Waters 510 pumps, Waters automated gradient controller and a Waters 712 wisp (Waters, Milford, MA).

Example 1

1(a): Preparation of neutralized Tryptorelin in presence of hydroxylapatite

200 mg of Hydroxyapatite (HAP) (American International Chemical, Natick, MA having particle size 2 μm) was suspended in water. 100 mg of the acetate salt of pyroGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2 (Tryptorelin, Kinerton, Dublin, Ireland) was dissolved in 1 ml of water and this solution was added to the suspension of HAP. The pH of the slurry was brought to about 7-8

by adding 1N $NaHCO_3$ dropwise. The precipitate was left stirring for about 2 hrs. The precipitate was collected by centrifugation. The precipitate was suspended in water and lyophilized.

Peptide content by nitrogen analysis = 23.6% and by HPLC= 22.1%.

1(b): Preparation of neutralized polyvinyl alcohol (PVA) solution

Commercially available PVA has pH lower than 5, due to the presence of hydrolysis product of poly(vinylacetate) from which PVA is prepared. The PVA solution was cleaned by preparing a concentrated solution in water, neutralizing with $NaHCO_3$ solution, dialyzing against de-ionized water. The neutralized PVA was precipitated in acetone, filtered and vacuum dried.

1(c): Preparation of p(dl-lactic acid) microspheres

1 g of p(dl-lactic acid) available from (Pharma-Biotech, Zl de Signes, BP 707, 83030 Toulon Cedex-9, France) (Mn= 32K, Mw= 54.4K) was dissolved in 10 ml DCM and 100 mg of the above product was suspended in the solution. The solution was cooled in an ice-bath and was dispersed in 100 ml of 1% pre-cooled PVA (polyvinyl alcohol) solution using a Polytron homogenizer (Kinematica, Switzerland). DCM was rotovaped and the microspheres were collected by centrifugation. The particles were suspended in water and lyophilized. Peptide content determined by nitrogen analysis was 2% (calculated 2.2%).

1(d): Preparation of neutralized Tryptorelin in presence of HAP

To 500 mg of acetate salt of pyroGlu-His-Trp-Ser-Tyr-DTrp-Leu-Arg-Pro-Gly-NH_2 (Kinerton, Dublin, Ireland) dissolved in 5 ml of water was added 200 mg of HAP. The pH of the solution was brought up to 7-8 using 1N $NaHCO_3$. The solution was left standing for about 2 hrs. and the precipitate was collected by centrifugation, and suspended in water and lyophilized. Peptide content by nitrogen analysis = 58.9%.

1(e): Preparation of microspheres containing 1(c)

Microspheres were prepared by employing the same procedure as 1(b). Peptide content 4.9%.

1(f): Co-precipitation of Tryptorelin and Calcium Phosphate monobasic

A solution of 100 mg of $CaHPO_4$ (Aldrich Chemicals, St. Louis, MO) and 100 mg of the acetate salt of pyroGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-

NH_2 (Kinerton, Dublin, Ireland) in water was prepared. The pH of the solution was brought to about 7 using 1N $NaHCO_3$ and left for about 24 hrs. for the completion of the precipitate. The precipitate was centrifuged, collected, suspended in water and lyophilized. Peptide content determined by HPLC method was 49.4%.

1(g): In-Vivo testing of 1(b) and 1(d) in rats

Formulations 1(b) & 1(d) were administered in male rats by IM injection at a dose of 300 μg of tryptorelin equivalent per rat, as a dispersion of the microspheres in 1% (w/v) Tween 20® (Aldrich Chemicals, St. Louis, MO) and 2% (w/v) carboxymethyl cellulose (Aldrich Chemicals, St. Louis, MO). The testosterone response was monitored by RIA: 50μL of the blood sample, 200μL of 125I-testosterone and 200μL of antiserum were poured into tubes which were shaken and incubated for 2 hrs. at 37°C. The immunoprecipitant reagent (1ml) was added to each tube and all the tubes were incubated for 15 minutes at room temperature. The supernatent was eliminated after centrifugation and the radioactivity was measured with LKB Wallace gamma counter. The plasma testosterone levels are shown below.

Table 1

Plasma testosterone response (ng/ml) to IM injection of 300 μg of Tryptorelin equivalent/rat.

Sample	6 h	Day 2	Day 3	Day 5	Day 10	Day 15	Day 23	Day 30	Day 37
1(b)	5.37	4.09	0.74	0.45	0.30	0.31	0.90	0.61	0.81
1(d)	5.32	3.58	1.04	0.29	0.38	0.56	0.80	0.75	0.72

Example 2

2(a): Preparation of water-insoluble salts of peptides with carboxylated p(dl-LGA)

Water insoluble salts of peptides with carboxy functionalized PLGA were prepared as described in US Patent No. 5,672,659 the teachings of which are incorporated herein by reference.

In a typical experiment 4 g of p(dl-lactide-co-glycolide) having Mn= 5560 and Mw= 12200, acid and polymer composition 70/30 dl-lactide/glycolide, prepared using 2% malic acid was dissolved in acetone. 0.73 ml 1N $NaHCO_3$ was added and stirred. The acetate salt of pyroGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2 (Kinerton, Dublin, Ireland) (0.64g) was dissolved in 2 ml water and was added to the polymer solution. The solution was stirred for about 2 hrs and precipitated in 400 ml cold water kept at about 4-6° C. Peptide content determined by nitrogen analysis was 9.8%.

2(b): Preparation of microspheres of 2(a)

1.5 g of the above vacuum dried complex was dissolved in 15 ml of DCM. The DCM solution was cooled in an ice-bath along with 150 ml of 1% PVA solution prepared from pure PVA as described above in Example 1(b). The DCM solution was slowly added to the PVA solution while it was being dispersed using a Polytron Homogenizer. The DCM was evaporated off, and the microspheres were collected by centrifugation. The microspheres were suspended in water and lyophilized. Peptide content by nitrogen analysis was 8.4%.

2(c): Preparation of dioctylsulfosuccinate of a Somatostatin analogue

To 100 mg of the somatostatin analogue [4-(2-hydroxyethyl)-1-piperazinylacetyl-D-cyclo(Cys-Tyr-D-Trp-Lys-Abu-Cys)-Thr-NH_2 acetate (Kinerton, Dublin, Ireland) dissolved in 3 ml of water was added 80 mg of sodium dioctylsulfosuccinate (Aldrich Chemicals, St. Louis, MO) dissolved in 4 ml of water. The precipitated peptide salt was collected by centrifugation, suspended in water and lyophilized. Peptide content by nitrogen analysis= 47.3%.

2(d): Preparation of p(dl-LGA) microspheres containing dioctylsulfosuccinate of a Somatostatin analogue

1 g p(dl-LA) was dissolved in 10 ml DCM. 150 mg of the [4-(2-hydroxyethyl)-1-piperazinylacetyl-D-cyclo(Cys-Tyr-D-Trp-Lys-Abu-Cys)-Thr-NH_2 (Kinerton, Dublin, Ireland) dioctylsulfosuccinate salt prepared in example 2(c) was added to the polymer solution. The mixture was sonicated to obtain a solution. This solution was cooled in an ice-bath, and was added to a pre-cooled 1% neutralized PVA solution, having pH=7, under stirring using a Polytron

Homogenizer. DCM was rotovaped off. Microparticles were filtered, washed with water, and dried under vacuum. Nitrogen analysis gave a peptide content of 7%.

2(e): In-vivo testing of 2(b) in rats

Formulation 2(b) was administered in male rats by IM injection at a dose of 300 μg of tryptorelin per rat, as a dispersion of the microspheres in 1% (w/v) Tween 20® and 2% (w/v) carboxymethyl cellulose. The testosterone response was monitored by RIA as described hereinabove. The plasma testosterone levels are shown below in Table 2.

Table 2

Plasma testosterone response (ng/ml) to IM injection of 300 μg of tryptorelin equivalent/rat.

Sample	Day 2	Day 5	Day 10	Day 15	Day 26	Day 36	Day 46
2(b)	3.98	1.04	0.63	0.76	0.60	0.37	0.86

CLAIMS

What is claimed is:

1. A process for preparing polymer microspheres comprising a polymer and a peptide, which comprises the steps of:

neutralizing a peptide salt with a weak base in an aqueous medium wherein said medium comprises a suspension of hydroxyapatite or a solution of calcium mono-hydrogen phosphate to form a precipitate;

isolating the precipitate;

suspending the precipitate in an organic solvent, which comprises a polymer dissolved therein to form a suspension;

dispersing the suspension in an aqueous solution of a surfactant; and

evaporating the organic solvent to isolate the polymer microspheres.

2. A process according to claim 1, comprising the additional step of dissolving the peptide salt in a minimum of water before neutralizing the peptide salt.

3. A process according to claim 2, wherein the surfactant is one or more of sodium oleate, sodium stearate, sodium laurylsulphate, a poly(oxyethylene) sorbitan fatty acid ester, polyvinylpyrrolidine, polyvinyl alcohol, carboxymethyl cellulose, lecithin, gelatin or hyaluronic acid.

4. A process according to claim 3, wherein the surfactant is polyvinyl alcohol and the pH of the polyvinyl alcohol is 6.5-7.5.

5. A process according to claim 4, wherein the pH of the polyvinyl alcohol is 6.9-7.1.

6. A process according to claim 5, wherein the organic solvent is dichloromethane, chloroform or ethyl acetate.

7. A process according to claim 6, wherein the organic solvent is dichloromethane and the concentration of the polymer in the organic solvent is 0.5% to 30% by weight.

8. A process according to claim 7, wherein the concentration of the polymer in dichloromethane is 0.5% to 10% by weight.

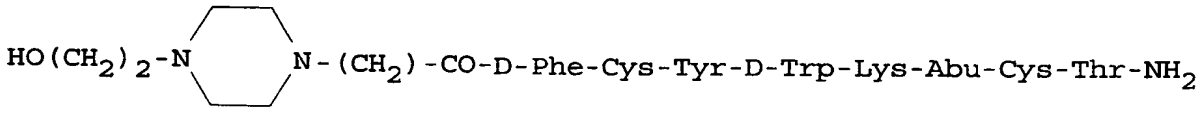
9. A process according to claim 8, wherein the peptide is growth hormone releasing peptide, luteinizing hormone-releasing hormone, somatostatin, bombesin, gastrin releasing peptide, calcitonin, bradykinin,

galanin, melanocyte stimulating hormone, growth hormone releasing factor, amylin, tachykinins, secretin, parathyroid hormone, enkephalin, endothelin, calcitonin gene releasing peptide, neuromedins, parathyroid hormone related protein, glucagon, neurotensin, adrenocorticothrophic hormone, peptide YY, glucagon releasing peptide, vasoactive intestinal peptide, pituitary adenylate cyclase activating peptide, motilin, substance P, neuropeptide Y, or TSH or an analogue or a fragment thereof or a pharmaceutically acceptable salt thereof.

10. A process according to claim 9, wherein the peptide is the LHRH analogue of the formula pyroGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2.

11. A process according to claim 10, wherein the polymer is polylactide-co-glycolide, polycaprolactone or polyanhydride or a copolymer or blends thereof.

12. A process according to claim 9, wherein the peptide is selected from the group of somatostatin analogues consisting of H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-NH_2,



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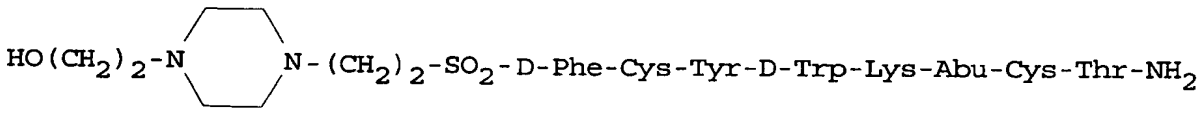

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13. A process according to claim 12, wherein the polymer is polylactide-co-glycolide, polycaprolactone or polyanhydride or a copolymer or blends thereof.

14. A polymer microsphere made according to the process of claim 1.

15. A polymer microsphere made according to the process of claim 11.

16. A polymer microsphere made according to the process of claim 13

17. A process for preparing polymer microspheres and nanospheres comprising a polymer and a peptide, which comprises the steps of:

dissolving a salt of a peptide complexed with an anionically or cationically functionalized biodegradable polyester in an organic solvent to form a solution;

dispersing the solution in an aqueous solution of a surfactant; and

evaporating the organic solvent to isolate the polymer microspheres and nanospheres.

18. A process according to claim 17, wherein the anionically functionalized biodegradable polyester is functionalized with an anionic moiety selected from the group consisting of carboxylate, phosphate and sulfate and the cationically functionalized biodegradable polyester is functionalized with a cationic moiety selected from the group consisting of amino, amidino, guadino, ammonium, cyclic amino groups and nucleic acid bases.

19. A process according to claim 18 wherein the organic solvent is dichloromethane, chloroform or ethyl acetate.

20. A process according to claim 19, wherein the organic solvent is dichloromethane and the concentration of the polymer in the dichloromethane is 0.5% to 30% by weight.

21. A process according to claim 20, wherein the concentration of the polymer in the dichloromethane is 0.5% to 10% by weight.

22. A process according to claim 21, wherein the surfactant is one or more of sodium oleate, sodium stearate, sodium laurylsulphate, a poly(oxyethylene) sorbitan fatty acid ester, polyvinylpyrrolidine, polyvinyl alcohol, carboxymethyl cellulose, lecithin, gelatin or hyaluronic acid.

23. A process according to claim 22, wherein the surfactant is polyvinyl alcohol and the pH of polyvinyl alcohol is 6.5-7.5.

24. A process according to claim 23, wherein the pH of polyvinyl alcohol is 6.9-7.1.

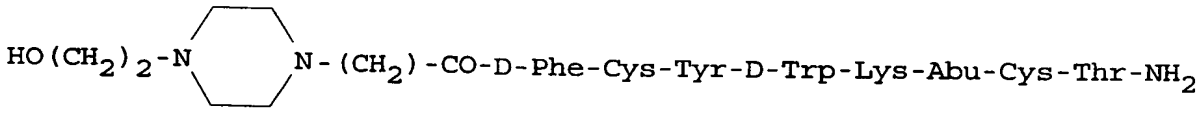
25. A process according to claim 24, wherein the peptide is growth hormone releasing peptide, luteinizing hormone-releasing hormone, somatostatin, bombesin, gastrin releasing peptide, calcitonin, bradykinin, galanin, melanocyte stimulating hormone, growth hormone releasing factor, amylin, tachykinins, secretin, parathyroid hormone, enkephalin, endothelin, calcitonin gene releasing peptide, neuromedins, parathyroid hormone related protein, glucagon, neurotensin, adrenocorticothrophic hormone, peptide YY,

glucagon releasing peptide, vasoactive intestinal peptide, pituitary adenylate cyclase activating peptide, motilin, substance P, neuropeptide Y, or TSH or an analogue or a fragment thereof or a pharmaceutically acceptable salt thereof.

26. A process according to claim 25, wherein the peptide is the LHRH analogue of the formula pyroGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2.

27. A process according to claim 26, wherein the polymer is polylactide-co-glycolide, polycaprolactone or polyanhydride or a copolymer or blends thereof.

28. A process according to claim 25, wherein the peptide is selected from the group of somatostatin analogues consisting of H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-NH_2,



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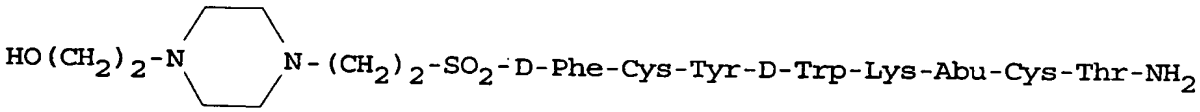

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29. A process according to claim 28, wherein the polymer is polylactide-co-glycolide, polycaprolactone or polyanhydride or a copolymer or blends thereof.

30. A polymer microsphere made according to the process of claim 17.

31. A polymer microsphere made according to the process of claim 27.

32. A polymer microsphere made according to the process of claim 29.

33. A process for preparing polymer microspheres and nanospheres comprising a polymer and a peptide, which comprises the steps of:

dissolving a salt of a peptide complexed with an anionic counterion in an organic solvent which is selected from the group consisting of dichloromethane, chloroform and ethyl acetate to form a solution;

dispersing the solution in a surfactant; and

evaporating the organic solvent to isolate the polymer microspheres and nanospheres.

34. A process according to claim 33, wherein the anionic counterion is dioctylsulfosuccinate, dodecylsulfate, tannate, pamoate, alginate, cyclodextrin sulfate, cyclodextrin phosphate, bisphosphonate or inisitol phosphate.

35. A process according to claim 34 wherein the organic solvent is dichloromethane.

36. A process according to claim 35, wherein the concentration of the polymer in dichloromethane is 0.5% to 30% by weight.

37. A process according to claim 36, wherein the concentration of the polymer in dichloromethane is 0.5% to 10% by weight.

38. A process according to claim 37, wherein the surfactant is one or more of sodium oleate, sodium stearate, sodium laurylsulphate, a poly(oxyethylene) sorbitan fatty acid ester, polyvinylpyrrolidine, polyvinyl alcohol, carboxymethyl cellulose, lecithin, gelatin or hyaluronic acid.

39. A process according to claim 38, wherein the surfactant is polyvinyl alcohol and the pH of polyvinyl alcohol is 6.5-7.5.

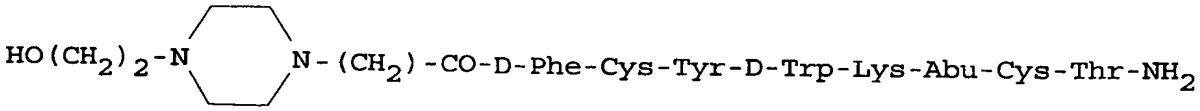
40. A process according to claim 39, wherein the pH of polyvinyl alcohol is 6.9-7.1.

41. A process according to claim 40, wherein the peptide is growth hormone releasing peptide, luteinizing hormone-releasing hormone, somatostatin, bombesin, gastrin releasing peptide, calcitonin, bradykinin, galanin, melanocyte stimulating hormone, growth hormone releasing factor, amylin, tachykinins, secretin, parathyroid hormone, enkephalin, endothelin, calcitonin gene releasing peptide, neuromedins, parathyroid hormone related protein, glucagon, neurotensin, adrenocorticothrophic hormone, peptide YY, glucagon releasing peptide, vasoactive intestinal peptide, pituitary adenylate cyclase activating peptide, motilin, substance P, neuropeptide Y, or TSH or an analogue or a fragment thereof or a pharmaceutically acceptable salt thereof.

42. A process according to claim 41, wherein the peptide is the LHRH analogue of the formula pyroGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2.

43. A process according to claim 42, wherein the polymer is polylactide-co-glycolide, polycaprolactone or polyanhydride or a copolymer or blends thereof.

44. A process according to claim 41, wherein the peptide is selected from the group of somatostatin analogues consisting of H-D-β-Nal-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr-NH_2,



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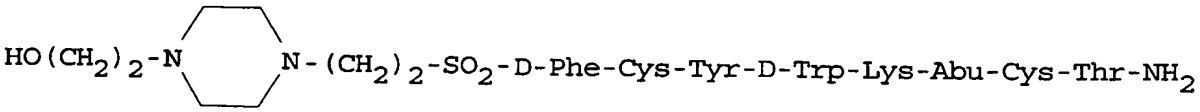

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45. A process according to claim 44, wherein the polymer is polylactide-co-glycolide, polycaprolactone or polyanhydride or a copolymer or blends thereof.

46. A polymer microsphere made according to the process of claim 33.

47. A polymer microsphere made according to the process of claim 43.

48. A polymer microsphere made according to the process of claim 45.